

Angiocrine signaling in sinusoidal homeostasis and liver diseases

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Summary

The hepatic sinusoids are composed of liver sinusoidal endothelial cells (LSECs), which are surrounded by hepatic stellate cells (HSCs) and contain liver-resident macrophages called Kupffer cells, and other patrolling immune cells. All these cells communicate with each other and with hepatocytes to maintain sinusoidal homeostasis and a spectrum of hepatic functions under healthy conditions. Sinusoidal homeostasis is disrupted by metabolites, toxins, viruses, and other pathological factors, leading to liver injury, chronic liver diseases, and cirrhosis. Alterations in hepatic sinusoids are linked to fibrosis progression and portal hypertension. LSECs are crucial regulators of cellular crosstalk within their microenvironment via angiocrine signaling. This review discusses the mechanisms by which angiocrine signaling orchestrates sinusoidal homeostasis, as well as the development of liver diseases. Here, we summarise the crosstalk between LSECs, HSCs, hepatocytes, cholangiocytes, and immune cells in health and disease and comment on potential novel therapeutic methods for treating liver diseases.

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Introduction

The microvascular circulation system in the liver that drains blood from the portal vein and hepatic artery to the liver parenchyma is called the liver sinusoid. The liver sinusoid is composed of liver sinusoidal endothelial cells (LSECs), a type of highly differentiated endothelial cell (EC) that is phenotypically different from other ECs. LSECs are the most abundant non-parenchymal liver cells, representing approximately 15–20% of the total number of liver cells,¹ and are characterized by transcellular pores and a lack of basement membrane (Fig. 1A). LSECs can be divided into periportal LSECs (zone 1), mid-zonal LSECs (zone 2), and pericentral LSECs (zone 3), and more than two-thirds of LSEC genes are differentially expressed along the portal-central axis of the liver lobule, indicating significant heterogeneity within the cell population.² Representative LSEC zonation markers identified by immunohistochemistry analysis, single-cell RNA sequencing (scRNA-seq), or, more recently, spatial transcriptomic studies, are summarized in Fig. 1B.^{2–8} Studies have revealed a complicated role of LSECs in physiological stages and liver diseases.^{9–11} LSECs can secrete a broad spectrum of paracrine factors, termed angiocrine signaling, through which LSECs interact with surrounding cells

comprising the sinusoidal niche, including hepatocytes, hepatic stellate cells (HSCs), Kupffer cells (KCs), and other circulatory immune cells.¹² This cellular crosstalk within the sinusoidal niche sustains normal sinusoidal functions called sinusoidal homeostasis, which is vital for maintaining liver homeostasis.

LSECs and sinusoidal niches are usually the first line of defense against pathological circulatory factors such as metabolites, toxins, and viruses.¹³ Upon injury, LSECs can gradually lose their specialized phenotype, marked by the loss of fenestrae and the development of a basement membrane (Fig. 1A).^{11,14,15} This pathological change is called sinusoidal capillarization, which is a typical phenotypic change accompanied by a significant functional switch in LSECs (Fig. 2).^{12,16} Capillarized LSECs change their angiocrine pattern, with increased release of vasoconstrictors, proinflammatory, profibrotic, and prothrombotic factors,^{9,17} which further promote disease progression and are closely associated with the pathogenesis of a broad spectrum of liver diseases.¹ In this review, we will discuss the mechanisms by which angiocrine signaling orchestrates sinusoidal homeostasis and how impaired angiocrine signaling contributes to liver diseases.

Keywords: liver sinusoidal endothelial cells; hepatic stellate cells; Kupffer cells; sinusoidal capillarization; liver regeneration; alcoholic liver disease; metabolic dysfunction-associated steatotic liver disease; NAFLD; NASH; MASLD; MASH; liver fibrosis; portal hypertension.

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Keypoints

- Liver sinusoids are composed of liver sinusoidal endothelial cells (LSECs), a type of highly differentiated endothelial cell characterized by transcellular pores and a lack of basement membrane.
- LSECs secrete a broad spectrum of paracrine factors that drive local cell crosstalk via angiocrine signaling and have a versatile effect on the regulation of hepatic blood microcirculation, the immune response, liver metabolism, and liver regeneration.
- Through angiocrine signaling, LSECs interact with surrounding cells comprising the sinusoidal niche, including hepatocytes, hepatic stellate cells, Kupffer cells, and other circulatory immune cells, to maintain sinusoidal homeostasis.
- Injured LSECs become capillarized, characterized by the loss of transcellular pores and disrupted angiocrine signaling, which results in dysregulated sinusoidal homeostasis and promotes the pathogenesis and progression of liver diseases.
- Dysfunctional LSECs and angiocrine signaling might be potential therapeutic targets for restoring sinusoidal homeostasis and treating liver diseases.

Angiocrine signaling in sinusoidal homeostasis and upon injury

Several cell types are associated with sinusoid homeostasis, including LSECs, HSCs, KCs, hepatocytes, and other peripheral immune cells, such as neutrophils and lymphocytes.¹⁸ The hepatocytes are separated from LSECs by the space of Disse, the 500–1,000 nm perisinusoidal space where HSCs reside. In contrast, liver-resident KCs and circulatory immune cells are located in the lumen of sinusoids and can infiltrate the liver parenchyma through the sinusoidal wall.^{10,19} Sinusoidal homeostasis is maintained through crosstalk between these cell types, and LSECs and angiocrine signaling play critical roles in this sophisticated intercellular communication through the release of various kinds of chemokines, trophogens, growth factors, and other molecules^{20–22} (Fig. 2). Angiocrine signaling is essential for liver development, zonation, immune response, and regeneration. Here, we summarize the angiocrine signaling between LSECs and other cells comprising the sinusoidal niche.

Hepatocytes

Hepatocytes are the major parenchymal cells of the liver and are arranged in hexagonal liver lobules. Hepatocytes have a lobular distribution along the portal-central axis, and hepatocytes in different zones exhibit distinct features.²³ Although hepatocytes are not in direct contact with LSECs, the zonation and function of hepatocytes are regulated by angiocrine signaling derived from LSECs. Hepatocytes can regulate the proliferation of LSECs via GATA3 and Ramp2.²⁴ In turn, angiocrine Wnt signaling is required to establish and maintain the pericentral functional pattern of hepatocytes in mice, as endothelial-specific inhibition of Wnt signaling is associated with disrupted pericentral hepatocyte zonation.^{25,26} However, central vein ECs are a major source of the Wnt signaling activator Rspodin 3 in the liver.²⁷ Therefore, the extent to which angiocrine signaling from LSECs plays a role in hepatocyte zonation remains to be identified.

Angiocrine signaling in LSECs also has a regulatory effect on hepatocyte metabolic functions. Bone morphogenetic protein (BMP)-2 and BMP-6 released by LSECs affect hepcidin expression in hepatocytes, which is essential for iron sensing and hepatic iron homeostasis, while LSEC-secreted transforming growth

factor- β (TGF- β) regulates BMP-2 secretion by LSECs via auto-crine signaling.^{28–30} *In vitro*, overexpression of the transcription factor c-Maf in human umbilical vein ECs induces an LSEC-like phenotype and helps sustain the expression of CYP1A2 in primary adult human hepatocytes and the secretion of albumin in a co-culture system, but this interaction needs to be further validated in *bona fide* LSECs and hepatocytes *in vivo*.³¹ Consistently, a recent study using a humanized mouse liver model further revealed that LSECs can regulate bile acid and cholesterol metabolism in hepatocytes through Wnt2 secretion.³² These findings suggest a vital role for LSECs in the regulation of hepatocyte zonation and function via angiocrine signaling. Furthermore, LSEC-derived angiocrine signaling modulates hepatocyte proliferation and liver regeneration, which is discussed in a subsequent section on “Angiocrine signaling in liver regeneration and diseases”.

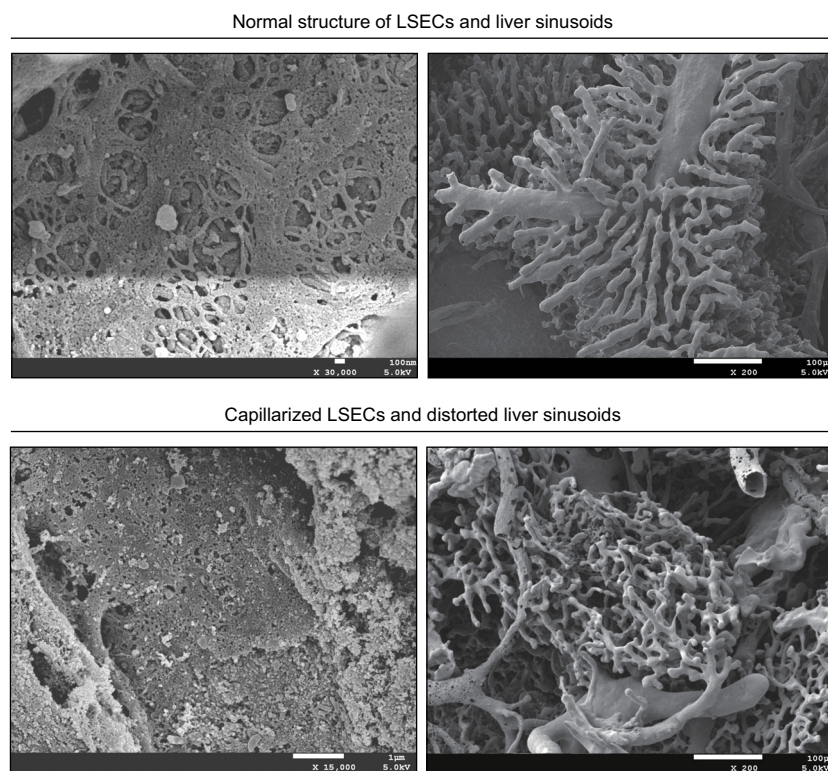
Cholangiocytes

Cholangiocytes represent less than 5% of the cell population in the liver. They line the bile ducts and are responsible for bile maturation and transportation. Notably, the crosstalk between LSECs and cholangiocytes has been poorly studied. The Notch signaling pathway is crucial for biliary development and repair, and LSECs can secrete ligands of the Notch signaling pathway.^{33,34} Consistent with these findings, hepatobiliary organoids exhibit improved bile duct structure in the presence of LSECs.³⁵ However, whether this effect depends on LSEC-derived Notch ligands has yet to be identified. More studies are needed to elucidate the interaction between LSECs and cholangiocytes during liver homeostasis and disease.

Hepatic stellate cells

HSCs are the major source of myofibroblasts, the primary producers of the hepatic extracellular matrix (ECM) during liver disease. It has become clear that LSECs and HSCs have intimate crosstalk.^{14,36} Co-culture experiments demonstrated that normal LSECs can suppress HSC activation and stimulate the reversion of activated HSCs back to a quiescent state,^{37,38} suggesting the anti-fibrotic role of healthy LSECs. The anti-fibrotic effect of LSECs is nitric oxide (NO) dependent and is mainly mediated by the vascular endothelial growth factor (VEGF) signaling pathway, which is diminished in capillarized

A



B

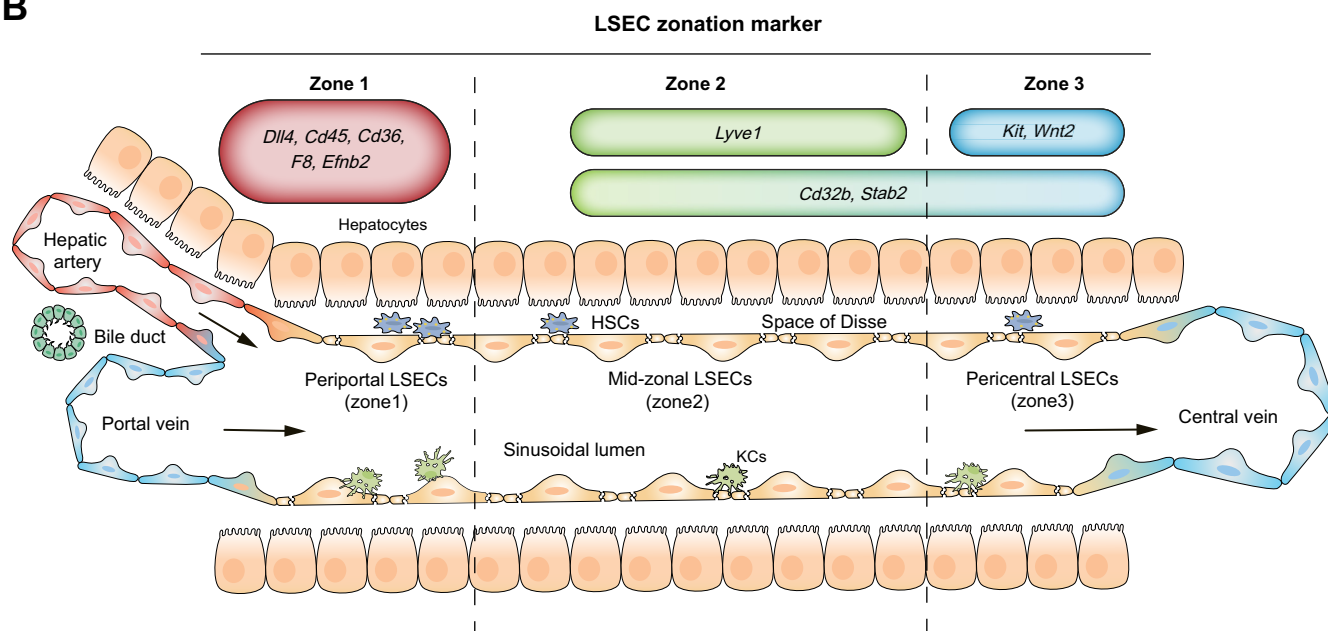


Fig. 1. Structures of normal and injured LSECs and zonation of LSECs across the hepatic sinusoid in healthy liver. (A) Upper left: typical SEM image of normal rat LSECs. The fenestrae are organized in sieve plates. Upper right: typical SEM image of the normal rat liver sinusoid cast. Liver sinusoids start from the portal vein and have a well-organized distribution. Bottom left: typical SEM image of capillarized rat LSECs. The number and size of fenestrae are significantly decreased. Bottom right: typical SEM image of the liver sinusoid cast in a cirrhotic rat liver. The structure of the liver sinusoids is distorted and narrowed. Courtesy of our group. (B) LSECs are classified into periportal (zone 1), mid-zonal (zone 2), and pericentral (zone 3) LSECs according to their location and diverse gene expression patterns. *Dll4*, *Cd45*, *Cd36*, *F8*, and *Efnb2* are highly expressed in periportal LSECs, *Lyve-1* is highly expressed in mid-zonal LSECs, and *Wnt2* and *Kit* are highly expressed in pericentral LSECs. *Cd32b* and *Stab2* are highly expressed in both mid-zonal and pericentral LSECs. Arrows indicate the direction of the blood flow. LSECs, liver sinusoidal endothelial cells; SEM, scanning electron microscopy.

LSECs.³⁸ Similarly, other angiocrine signaling pathways also contribute to HSC deactivation. Kruppel-like factor 2 (KLF2) is an important vasoprotective factor that can induce LSEC

expression of vasodilators and endothelial nitric oxide synthase (eNOS). KLF2-NO-guanylate cyclase (GC) angiocrine signaling contributes to the deactivation of HSCs by LSECs *in vitro*.³⁹

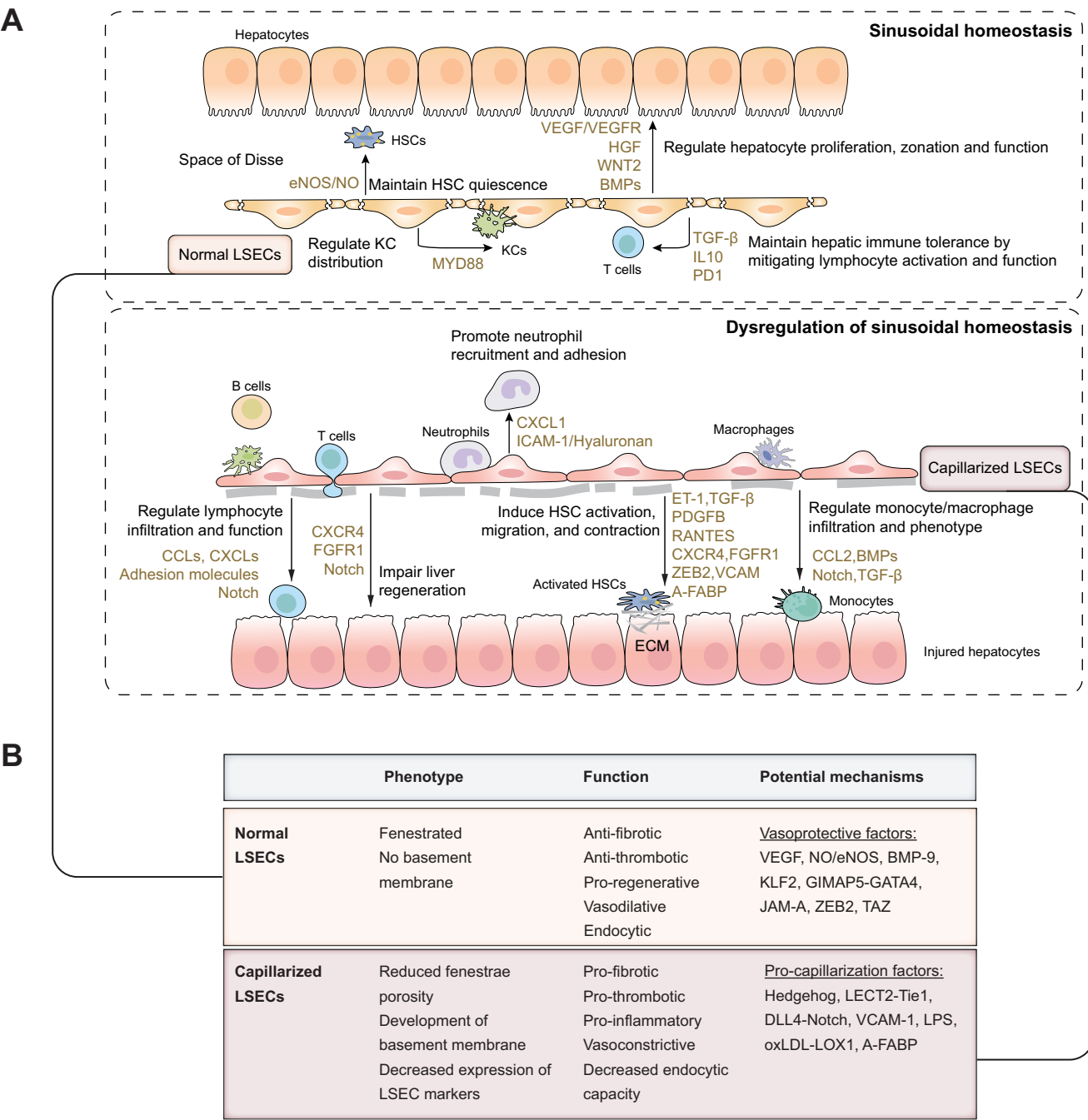


Fig. 2. The central role of LSECs in sinusoidal homeostasis, the phenotypical and functional alterations of capillarized LSECs, and potential mechanisms. (A) The sinusoidal niche includes LSECs, HSCs, hepatocytes, and immune cells. Sinusoidal homeostasis relies on orchestrated crosstalk among these cells. LSECs are the central cells in the sinusoidal niche and play a pivotal role in maintaining sinusoidal homeostasis. Healthy LSECs can maintain HSC quiescence and regulate hepatocyte proliferation, zonation, and function. Moreover, healthy LSECs regulate KC distribution and maintain hepatic immune tolerance by mitigating lymphocyte activation and function. However, upon liver injury, LSECs become capillarized and acquire profibrotic and proinflammatory phenotypes. Injured LSECs can induce HSC activation, migration, and contraction. Moreover, injured LSECs promote immune cell infiltration and activation. Furthermore, injured LSECs impair liver regeneration by inhibiting hepatocyte proliferation. (B) Phenotypical and functional alterations of capillarized LSECs compared to normal LSECs and potential mechanisms driving capillarization. HSCs, hepatic stellate cells; KCs, Kupffer cells; LSECs, liver sinusoidal endothelial cells.

Reciprocally, the vasoprotective factor BMP9 (bone morphogenetic protein 9) secreted by HSCs can preserve LSEC differentiation, and the deletion of *Bmp9* induces LSEC capillarization and perisinusoidal fibrosis *in vivo*.⁴⁰

In general, LSEC-derived angiocrine signaling is a potent regulator of HSC fate and thus influences the course of liver fibrosis.³⁷ The detailed crosstalk between LSECs and HSCs during liver injury or fibrosis is discussed later in Section 3.

ScRNA-seq analysis revealed heterogeneity of HSC subsets,^{41–45} and whether LSECs activate all HSCs or a specific profibrotic subset of HSCs needs to be investigated. Moreover, additional studies are needed to clarify the relationships among LSECs, portal fibroblasts, and mesothelial cells, the other two sources of myofibroblasts.⁴⁶

Immune cells

LSEC-mediated angiocrine signaling plays a central role in the immune homeostasis of hepatic sinusoids. In the context of tissue injury and inflammation, circulating immune cells are recruited to organs by chemoattractants, followed by a typical stepwise infiltration process, including rolling, adhesion to the vascular wall, crawling, and transmigration through the post-capillary venules.⁴⁷ In the liver, approximately 80% of leukocyte adhesion occurs in the hepatic sinusoid.⁴⁸ Rolling is not required in the hepatic sinusoid due to the low shear stress and narrow lumen.^{13,47,48} LSECs express an array of adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), mucosal addressin cell adhesion molecule 1, stabilin-2, and vascular adhesion protein 1 (VAP1), to guide leukocyte infiltration.^{49–52} Moreover, LSECs are the predominant source of the C-X-C motif chemokine ligand family (including CXCL1, 6, 8) in the liver^{53,54} and can secrete a wide variety of chemokines, such as C-C motif chemokine ligand (CCL) 2, CCL25, and C-X3-C motif chemokine ligand 1 (CX3CL1) upon injury.^{55–58} The dynamic crosstalk between LSECs and immune cells is critical for the recruitment and transendothelial migration of various immune cell subsets.⁵⁹

Macrophages

Macrophages represent the predominant immune cell type in the healthy liver.^{60,61} Hepatic macrophages originate from two dominant sources: liver-resident KCs located on the luminal side of the sinusoidal wall, and patrolling bone marrow-derived monocytes from peripheral circulation.⁶² Both KCs and monocyte-derived macrophages are distributed heterogeneously and play multifaceted roles in both liver homeostasis and diseases.^{63–66} Generally, KCs are the main hepatic macrophage population during homeostasis but they are depleted during major or sustained liver injury, wherein monocyte-derived macrophages accumulate in the liver, restoring the hepatic macrophage population.⁶⁷ As LSECs have direct membrane contact with KCs, their crosstalk is presumably essential for maintaining hepatic sinusoidal homeostasis. This section describes how LSEC-mediated angiocrine signaling impacts hepatic macrophage populations.

Physiologically, LSECs are involved in the development and perpetuation of KC identity,^{68,69} as well as the spatial polarization of KCs in the mouse liver via the MYD88-dependent signaling pathway.⁷⁰ This indicates that microbial signals from the gut-liver axis inform LSECs to organize the density and distribution of KCs.⁷⁰ Another example of pivotal LSEC-macrophage crosstalk is the “KC niche” which is defined by LSECs, HSCs, and hepatocytes that synergistically improve KC repopulation after KC loss.⁶⁹ Then, the specific role of LSECs in hepatic macrophage recruitment and differentiation was further explored. In an *in vivo* diphtheria toxin-mediated KC depletion mouse model, LSECs upregulated the expression of adhesion

molecules and chemokines that favored monocyte recruitment and transmigration.^{68,69} It was also reported that human LSECs facilitate the paracellular transmigration of monocytes after exposure to a senescence-associated secretory phenotype *in vitro*.⁷¹ Subsequently, LSECs secrete Notch ligands, TGF- β family ligands, and BMPs, which induce LXR α , RBPJ, and SMAD expression on infiltrated monocytes, resulting in monocyte differentiation toward a KC-like phenotype and maintenance of KC identity.^{68,69}

Under conditions of liver injury, the composition of hepatic macrophages undergoes drastic changes, with a prominent accumulation of monocyte-derived, functionally distinct macrophages in the liver that regulate inflammatory and fibrogenic responses.^{63,72} CD16⁺ monocytes are recruited by and transmigrated through LSECs in a CX3CL1/VAP1-dependent manner *in vitro*.⁵⁷ LSECs also secrete CCL2, which is regulated by p300 histone acetyltransferase and the bromodomain containing 4 (BRD4)/NF- κ B transcription activator complex, to recruit monocytes in the early phase of liver injury in mice.⁵⁸ LSEC-specific p300 deletion or *Ccl2* knockdown significantly reduces the liver recruitment of C-C motif chemokine receptor 2-expressing (CCR2⁺) monocytes.⁵⁸

LSECs may also directly impact monocyte and macrophage heterogeneity. *In vitro*, primary rat macrophages cultured with conditioned medium from LSECs isolated from cirrhotic rats are prone to develop a pro-inflammatory phenotype, indicating that the angiocrine factors derived from LSECs might impact the macrophage phenotype.⁷³ In another *in vitro* study using primary murine LSECs and monocytes, most monocytes that transmigrated through LSECs remained in the subendothelial space and obtained a macrophage-like phenotype and phagocytic function, while they suppressed T-cell activation and were unresponsive to lipopolysaccharide (LPS) induction. In contrast, approximately 10% of monocytes underwent “reverse transmigration” through LSECs and developed an immature dendritic cell phenotype with proinflammatory functions.⁷⁴ It seems that monocytes can differentiate into pro- and anti-inflammatory subtypes, and specific monocyte subsets are potential precursors of dendritic cells,⁷⁵ with LSECs potentially involved in this process. In turn, infiltrated monocytes, but not KCs, are required for endothelial survival and remodeling during liver regeneration,^{76,77} indicating that monocytes might also influence LSEC fate.

Taken together, these findings highlight the vital role of reciprocal interactions between LSECs and monocytes/macrophages in the context of liver development, injury, and inflammation. In this respect, it is important to understand and further study the effects of LSEC-mediated angiocrine signaling on the chronological and spatial distribution of liver macrophages during injury.

Neutrophils

LSEC-mediated angiocrine signaling also contributes to the recruitment and adhesion of neutrophils to the hepatic sinusoid. Neutrophils are considered proinflammatory cells and exacerbate tissue damage.⁶² More recent data have emphasized the contribution of neutrophils to ameliorating inflammation in the liver as well.⁷⁸ CXCL1, CXCL2, and CXCL5 in mice and CXCL8 in humans are major chemokines that recruit neutrophils to the liver.⁷⁹ Among the CXCLs that recruit

neutrophils, CXCL1, which is secreted by LSECs, is the best studied chemokine in liver diseases. During portal hypertension, elevated mechanical stretching leads to upregulated CXCL1 secretion via the Notch signaling pathway and Piezo channels in LSECs and subsequently increased neutrophil infiltration into the liver in mice.⁸⁰ In alcohol-related liver diseases, LSEC expression of CXCL chemokines is also epigenetically regulated by BRD4-NF- κ B-driven super-enhancer (a group of enhancers enriched for transcription factors)-promoter interactions.⁵⁴ In liver fibrosis, the expression of the glycolytic enzyme phosphofructokinase 1 isoform P in LSECs is upregulated in response to elevated tissue stiffness and leads to enhanced glycolysis. Glycolysis increases the transcription of CXCL1 through enlarged nuclear pores and NF- κ B translocation into the nucleus in mice.⁸¹ However, hepatocytes are another cellular source for CXCL1 production in high-fat diet plus alcohol-induced murine liver fibrosis.⁸² Thus, the cellular source of CXCL1 may change in response to different stimuli.

In addition to chemokine release for neutrophil recruitment, the adhesion of neutrophils is also pivotal for LSEC-mediated neutrophil infiltration. CD44 is a cell-surface receptor involved in cell interactions, adhesion, and migration. The interaction between CD44 expressed on recruited neutrophils and hyaluronan constitutively expressed on LSECs is required for neutrophil adhesion during systemic inflammation.⁸³ In contrast, the binding of integrin β 2 on neutrophils to sinusoidal ICAM-1 is required for neutrophil adhesion and crawling to LSECs during local inflammation.⁸⁴ Taken together, LSECs play an important role in the recruitment of neutrophils during liver injury, and the interaction between LSECs and neutrophils is context dependent. More studies are needed to dissect the detailed mechanisms of crosstalk between the two cell types in different liver disease settings.

Lymphocytes

Angiocrine signaling also maintains sinusoidal homeostasis by regulating lymphocyte recruitment. Lymphocytes consist of heterogeneous cell types – including T lymphocytes, B lymphocytes, innate lymphoid cells, such as natural killer cells, natural killer T cells and other types of non-conventional T cells (e.g. mucosal-associated invariant T cells) – with each kind of lymphocyte encompassing various subsets.⁶²

Lymphocyte recruitment, adhesion and transendothelial migration are regulated by LSEC-mediated angiocrine signaling. T lymphocytes can be classified into CD4⁺ T cells (also known as T helper cells or Th cells), which can assist in the maturation and activation of other immune cells, and CD8⁺ T cells (also known as cytotoxic T cells), which can directly kill other cells. These cells can differentiate into specialized subsets with different functions, such as regulatory T cells and memory T cells.⁶² Injured LSECs can secrete chemo-attractants, such as CCLs and CXCLs, contributing to the hepatic recruitment of lymphocytes.^{47,55,85,86} Moreover, the chemotactic effect of CXCL9 and CXCL12 on CD4⁺ T cells is enhanced in the presence of LSECs.⁸⁷ After recruitment, lymphocyte adhesion and transendothelial migration are mediated by the interaction between integrin on the surface of lymphocytes and adhesion molecules on LSECs, including VCAM-1, ICAM-1, mucosal addressin cell adhesion molecule 1, stabilin-2, and VAP1.^{13,50–52,88–90} Notably, these factors that

can activate transmigration are not solely produced by LSECs themselves. Mouse LSECs can also take up CXCL12 from the basolateral side and present it on the luminal side to induce CD4⁺ T cell transmigration,^{87,91} which indicates possible chemokine transfer within the sinusoidal niche during the recruitment of lymphocytes. Intriguingly, transmigration may not be indispensable for CD8⁺ T cells to modulate liver inflammation. In a mouse model of hepatitis B virus infection, instead of transmigration, the effector CD8⁺ T cells trapped by LSECs extended cytoplasmic protrusions through the pores on LSECs in search of antigens presented by adjacent infected hepatocytes and exerted cytotoxic effects.⁹²

In addition to its role in lymphocyte recruitment, adhesion, and transendothelial migration, LSEC-mediated angiocrine signaling also regulates hepatic immune tolerance to maintain sinusoidal homeostasis. Physiologically, LSECs contribute to maintaining hepatic immune tolerance by mitigating lymphocyte activation and function.^{93–95} Moreover, LSECs isolated from healthy human livers can induce T-cell apoptosis partially via TGF- β *in vitro*.⁹⁶ In addition, TGF- β has a greater effect on LSEC-induced immune tolerance, as LSECs promote the induction of regulatory T cells in a TGF- β -dependent manner.⁹⁷ Moreover, LSECs can suppress the proinflammatory secretion of Th1 and Th17 effector CD4⁺ T cells in a cell contact-mediated manner in mice, which requires interleukin- (IL-)10- and programmed death 1-dependent signaling pathways in LSECs.⁹⁸ Furthermore, LSECs induce CD8⁺ T-cell tolerance, which is characterized by reduced cytotoxicity.⁹⁵

In the case of liver injury, LSEC-mediated angiocrine signaling also has the potential to control the extent of the immune response. On the one hand, injured or infected LSECs can switch to an immunogenic phenotype and activate CD8⁺ T cells or Th1 cells through Toll-like receptor-mediated IL-12 secretion or direct cell contact during transmigration, respectively.^{89,99} LSECs also serve as a platform for CD4⁺-CD8⁺ T-cell crosstalk to cross-prime CD8⁺ T cells.¹⁰⁰ Furthermore, LSECs are involved in natural killer cell recruitment and priming in LPS-induced acute liver inflammation in mice.⁸⁶ On the other hand, LSECs can secrete ligands of the Notch signaling pathway and induce IL-10 expression in Th1 cells or suppress T-cell function by binding to activated T cells via lectin, both of which might be important for preventing an excessive immune response.^{33,101}

In general, LSEC-mediated angiocrine signaling plays a critical role in maintaining immune homeostasis in healthy or diseased livers by regulating lymphocyte recruitment, tolerance, and activation. Given that lymphocytes can differentiate into various specialized subsets with different functions, future work focusing on the precise regulation between LSEC-mediated angiocrine signaling and specific lymphocyte subsets could provide a better understanding of lymphocyte accumulation and its role in liver diseases.

Angiocrine signaling in liver regeneration and diseases

Liver regeneration

Unlike most other organs, the liver can regenerate and recover from damage. Liver regeneration is a complex process involving intricate and timely-orchestrated crosstalk between different

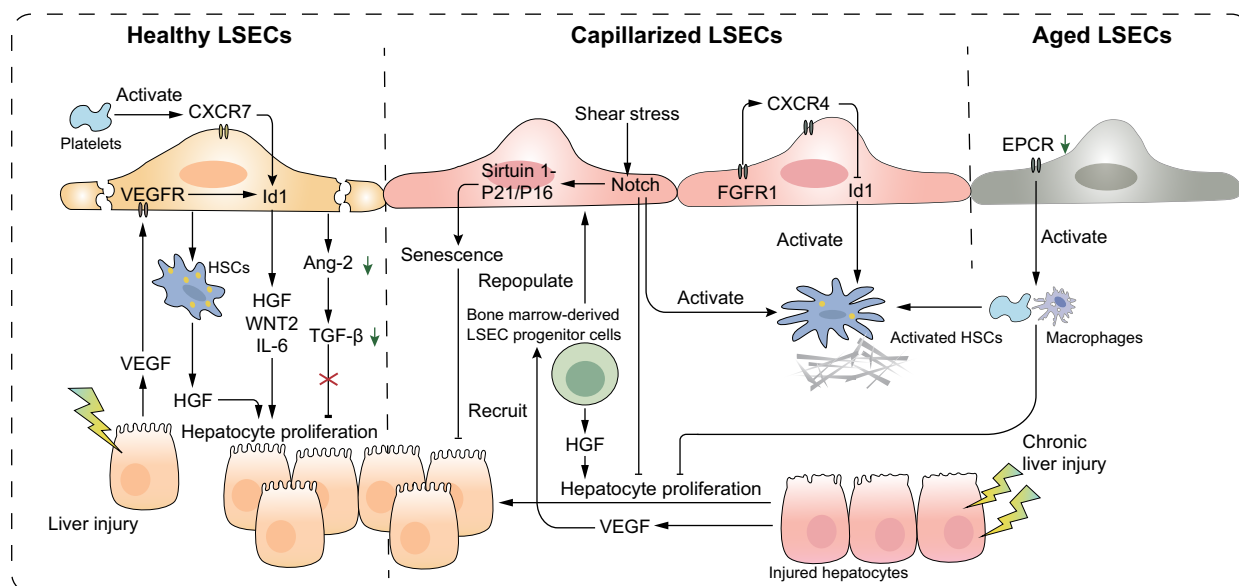


Fig. 3. The role of LSEC angiocrine signaling in liver regeneration. Under conditions of acute or mild liver damage, LSECs promote hepatocyte proliferation by increasing the secretion of WNT2, IL-6, and HGF through the VEGFR-Id1 signaling pathway. Moreover, the secretion of Ang-2 is decreased, leading to decreased TGF- β production and increased hepatocyte proliferation. Id1 can be activated by CXCR7, which is upregulated in LSECs during liver injury. Platelets can also activate CXCR7. In contrast, during chronic liver injury, CXCR4 expression in LSECs predominates over CXCR7 expression, which is mediated by FGFR1. CXCR4 overexpression inhibits Id1 expression and results in the activation of HSCs and impaired hepatocyte proliferation. LSEC senescence and aging also impair liver regeneration. Bone marrow-derived LSEC progenitor cells can be recruited to the sinusoidal niche by VEGF secreted by injured hepatocytes. These LSEC progenitor cells can repopulate injured LSECs and secrete HGF in favor of hepatocyte proliferation. HSCs, hepatic stellate cells; LSECs, liver sinusoidal endothelial cells.

cells. LSECs mediate angiocrine signaling and provide crucial cytokines and growth factors to initiate and support hepatocyte regeneration and sinusoidal homeostasis (Fig. 3).¹⁰²

The role of LSECs varies in different stages of liver regeneration. In the early stage of liver regeneration, Wnt proteins secreted by LSECs facilitate hepatocyte propagation and liver regeneration.^{103,104} Wnt is the ligand of the canonical β -catenin signaling pathway in hepatocytes and other liver cells and is involved in liver development, liver zonation, and liver regeneration.^{25,105} After partial hepatectomy in mice, a standard liver regeneration model, hepatic ECs promote liver regeneration through the secretion of Wnt proteins within 12 h after hepatectomy. In this process, Wnt2 from LSECs and central venous ECs is one of the major contributors to β -catenin activation and subsequent liver regeneration.¹⁰³ Specifically, a subset of c-kit⁺ LSECs was observed 1 day after partial hepatectomy and can induce hepatocyte proliferation through Wnt2-dependent angiocrine signaling in mice. This interaction is also found in carbon tetrachloride (CCl₄) or dimethylnitrosamine-induced mouse liver injury, while the pro-regenerative effect is abrogated after Notch activation in LSECs.¹⁰⁶ In the later stage, which is also called the angiogenic phase, LSECs proliferate and secrete hepatocyte growth factors (HGFs) and help to restore liver mass. Angiopoietin-2 (Ang-2) is another important LSEC-derived angiocrine factor that has a phase-specific effect on liver regeneration. In the early stages of liver regeneration, LSEC-derived Ang-2 expression is downregulated, leading to reduced TGF- β production and increased hepatocyte proliferation.¹⁰⁷ Conversely, increased Ang-2 expression in later stages promotes LSEC proliferation and VEGF receptor (VEGFR)-2 expression.¹⁰⁷ These findings suggest that LSEC-derived Ang-2 controls liver regeneration through both stimulatory and inhibitory effects.

VEGF is an important factor in liver regeneration and promotes liver regeneration in multiple ways. VEGF released by injured hepatocytes exerts a distinct effect by binding to VEGFR-1 and VEGFR-2 expressed on LSECs.¹⁰⁸ When binding to VEGFR-1, VEGF can promote the secretion of hepatocyte mitogenic factors such as HGFs and IL-6 from LSECs, which accelerates liver regeneration.¹⁰⁸ When binding to VEGFR-2, VEGF mainly triggers LSEC mitogenesis and increases the expression of several hepatotropic factors in specific subsets of LSECs throughout liver regeneration.^{108,109} These results indicate that VEGF might balance the pro-regenerative and pro-angiogenesis functions of LSECs.

Id1 is a downstream transcription factor of the VEGF-VEGFR2 signaling pathway that inhibits DNA binding and transcriptional activation. After acute liver damage, LSECs promote hepatocyte proliferation by increasing the secretion of Wnt2a and HGFs through the VEGFR2-Id1 signaling pathway.^{109,110} VEGFA can improve liver regeneration in the steatotic liver by activating the PI3K/AKT signaling pathway when combined with anti-sFlt1 treatment which increases VEGFA availability in the steatotic liver.¹¹¹ Studies have also revealed that during the later stages of liver regeneration, VEGF recruits bone marrow-derived endothelial sinusoidal progenitor cells to the site of injury to repopulate the sinusoidal endothelium. These cells are enriched with HGFs and promote hepatocyte proliferation.¹¹²

C-X-C motif chemokine receptors (CXCRs) regulate Id1, and CXCRs exhibit different endothelial expression patterns during liver regeneration. The balance between CXCR7 and CXCR4 can regulate liver regeneration in acute and chronic murine liver injury models. Activation of CXCR7 in LSECs induces Id1 expression, stimulating pro-regenerative angiocrine factors. However, in chronic liver injury models, the fibroblast

growth factor receptor 1 (FGFR1)-CXCR4 signaling pathway predominates over the CXCR7 pathway in LSECs, stimulating the activation of HSCs and promoting excessive ECM deposition over liver function restoration.^{113,114} Platelets can also activate CXCR7. Platelets adhere to LSECs in the injured liver and subsequently activate LSECs, stimulating the activation of CXCR7 and the secretion of pro-regenerating factors, such as IL-6 and Wnt2, eventually promoting hepatocyte proliferation.^{115,116} However, whether platelets adhering to LSECs can promote regeneration over fibrosis by altering the balance of CXCR7 and CXCR4 signaling remains unclear.

In addition, LSEC senescence and aging are involved in LSEC-mediated liver regeneration. Senescence is a stable growth arrest process accompanied by a proinflammatory secretome, chromatin remodeling, and metabolic reprogramming. LSEC senescence leads to LSEC capillarization and dysregulated LSEC zonation as a result of decreased expression of c-kit in the pericentral endothelium.^{117,118} Shear stress-induced activation of the endothelial Notch-Sirtuin 1-P21/P16 signaling pathway can promote LSEC senescence and impair sinusoidal homeostasis, enhance liver fibrosis and impede hepatocyte regeneration.^{117,119} Consistently, aging can disrupt the crosstalk between platelets and LSECs by suppressing the endothelial protein C receptor. Inhibition of the endothelial protein C receptor significantly dysregulates LSEC angiocrine signaling and sinusoidal homeostasis, characterized by the recruitment and activation of platelets and macrophages, the exacerbation of fibrosis, and the impairment of hepatocyte proliferation.¹²⁰ As aging reduces the regenerative capacity of the liver, studies on LSEC senescence might attract more attention to liver injury and regeneration.

The cell fate of LSECs is also crucial for restoring liver microarchitecture. Inhibited LSEC proliferation and increased LSEC apoptosis are associated with impaired liver regeneration in mice after partial hepatectomy.¹²¹ An in-depth examination of the cell landscape in CCl₄-induced liver injury revealed a unique process in which daughter hepatocytes from cell division align in the direction of the closest sinusoid, known as hepatocyte-sinusoid alignment, and a similar process is also observed in early hepatocellular carcinoma development.^{122,123} Hepatocyte-sinusoid alignment is a key mechanism for restoring liver microarchitecture, and LSECs are highly involved in this process. However, the molecular mechanism underlying this process is still elusive.

In summary, LSEC-mediated angiocrine signaling supplies essential cytokines and growth factors to initiate and enhance hepatocyte proliferation and maintain sinusoidal homeostasis during liver regeneration (Fig. 3). Some issues remain to be clarified, such as the crucial initial and stop signals that control angiocrine signaling, and the mechanism of shear stress-induced angiocrine signaling during liver regeneration. Additionally, the critical angiocrine signaling pathway that balances liver regeneration and fibrosis needs to be explored.

Alcohol-related liver disease (ALD)

The liver is involved in the metabolism of alcohol, and excessive alcohol consumption can lead to significant damage, in the form of hepatotoxicity, steatohepatitis, alcohol-associated hepatitis (AH) and/or cirrhosis. LSECs are directly exposed to alcohol in the sinusoids. Therefore, LSECs are especially

vulnerable to damage from alcohol intake. In patients with AH, LSEC dysfunction is indicated by dysregulation of a broad spectrum of soluble endothelial activation markers, including soluble CD146, ICAM-1, and VCAM-1.¹²⁴ Increased expression of endothelial activation markers is also associated with a worse prognosis in patients with AH, suggesting that endothelial dysfunction may play a role in AH progression.¹²⁵ Nevertheless, endothelial dysfunction might also be a consequence of hepatocyte death during AH, making their correlation with AH progression coincident. Therefore, whether there is a causal relationship between endothelial dysfunction and AH progression remains to be elucidated. CYP2E1 is a crucial enzyme in alcohol metabolism. CYP2E1 is upregulated in LSECs under alcohol intake. In turn, upregulated CYP2E1 causes HSP90 acetylation and eventually leads to LSEC dysfunction characterized by reduced NO production and proinflammatory angiocrine signaling *in vitro*.¹²⁶ It should be noted that hepatocytes have the highest CYP2E1 expression in the liver, are the main metabolizers of alcohol, and exhibit increased CYP2E1 expression under alcohol intake. Given the close proximity of hepatocytes and LSECs, the extent to which the CYP2E1 and ethanol metabolites of hepatocytes contribute to endothelial dysfunction remains to be determined. Moreover, reactive cholangiocytes promote liver angiogenesis via the Slit2-Robo1 signaling pathway in a chronic liver injury mouse model and are associated with disease severity in patients with ALD.¹²⁷ The above results indicate that alcohol damages LSECs.

Alcohol-mediated injury induces LSECs to secrete multiple chemokines to recruit immune cells, leading to enhanced hepatic inflammation. The CXCL family of chemokines is highly upregulated in patients with AH, and high levels of CXCL1, CXCL5, and CXCL6 correlate with worse clinical outcomes in these patients.¹²⁸ Consistently, dysfunctional LSECs culminate in dysregulated sinusoidal homeostasis and can cause the accumulation of neutrophils via CXCL1, CXCL6, and CXCL8.⁵⁴ The expression of multiple CXCL chemokines in LSECs from AH livers is regulated by a super-enhancer and the tumour necrosis factor- α (TNF α)/NF- κ B signaling pathway.⁵⁴ Moreover, ethanol induces the expression and translocation of high-mobility group box 1 (HMGB1) in hepatocytes. HMGB1 promotes the migration of HSCs and liver ECs to the site of injury and enhances inflammatory reactions.¹²⁹

In contrast, studies have suggested that endothelial signal transducer and activator of transcription 3 (STAT3) might be an anti-inflammatory molecule in ALD.⁹ Compared with wild-type mice, endothelial-specific *Stat3* knockout mice fed with ethanol exhibited exacerbated liver inflammation, sinusoidal apoptosis, and sinusoidal dysfunction, indicating that endothelial STAT3 has anti-inflammatory and anti-apoptotic effects on alcohol-related liver injury.¹³⁰ Similarly, LSECs isolated from ethanol-fed rats showed hampered STAT3 activation by IL6, suggesting defective endothelial STAT3 signaling during ALD.¹³¹ In summary, alcohol intake directly injures LSECs and disturbs sinusoidal homeostasis, and LSECs play multifaceted roles in the pathogenesis and progression of ALD.

Metabolic dysfunction-associated steatotic liver disease

Non-alcoholic fatty liver disease (or NAFLD) is characterized by liver steatosis and steatohepatitis and is often accompanied by

overweight and/or type 2 diabetes; thus, it is considered a metabolic disease.¹³² To better reflect the systemic nature of the disease, the new acronym MASLD, *i.e.* metabolic dysfunction-associated steatotic liver disease, has been introduced.¹³³ LSECs and angiocrine signaling are highly involved in the pathophysiology of MASLD (Fig. 4).¹³⁴ Under normal conditions, LSECs contribute to lipid exchange by transporting lipids either through fenestrae or by endocytosis.¹⁷ However, in the early stage of liver steatosis, lipid-induced NADPH oxidase 1 (NOX1) upregulation might hinder NO bioavailability and ultimately lead to endothelial dysfunction.¹³⁵ Similarly, oxidised low-density lipoprotein, which is involved in MASLD pathogenesis, triggers capillarization of human LSECs *in vitro* by binding to its receptor lectin-like oxidised LDL receptor 1 (LOX1).¹³⁶ LSEC capillarization is a sign of hepatic endothelial dysfunction. Capillarized LSECs exhibit decreased permeability and impaired lipid transfer, resulting in triglyceride and cholesterol accumulation in the liver.^{17,137} In addition, LSEC capillarization leads to the retention of chylomicron remnants in the blood, which might indirectly contribute to liver steatosis by triggering *de novo* lipogenesis in hepatocytes.^{17,137}

In addition to their role in lipid homeostasis, LSECs also regulate the immune response during MASLD. LSECs can play an anti-inflammatory role in the initial stages of MASLD by mitigating the activation of KCs.¹³⁸ LSECs treated with fatty acid *in vitro* exhibited decreased expression of proinflammatory CCL2, CXCL10, and CXCL16, which is associated with reduced monocyte migration.¹³⁹ Nevertheless, in the later stages of MASLD, dysfunctional LSECs acquire a proinflammatory phenotype and accelerate the hepatic inflammatory response.¹⁷ Its proinflammatory function is mainly characterized by the progressive upregulation of adhesion molecules at

the surface of LSECs, including ICAM-1 and VCAM-1, and other proinflammatory mediators, such as IL-6 and CCL2.^{140,141} In murine metabolic dysfunction-associated steatohepatitis (MASH) and liver fibrosis models, macrophages can regulate a subset of ECs (in the setting of one-time CCl₄ injection, VCAM-1⁺CTGF⁺ LSECs) with profibrotic phenotypes through the Yes-associated protein (YAP)/interferon signaling pathway and contribute to liver fibrosis.¹⁴² Nevertheless, clarification is required regarding which subgroup of ECs is predominant in interacting with macrophages during MASH. As a critical messenger for cell-to-cell crosstalk, extracellular vesicles (EVs) also contribute to the immune response in MASLD. Hepatocytes subjected to lipotoxic stress release EVs containing integrin β 1, which mediates the adhesion of monocytes to LSECs, leading to aggravated hepatic inflammation in MASLD.¹⁴³ Furthermore, in the human cirrhotic liver and minipig MASH model, the abnormal activation of histone deacetylase 2 (HDAC2) and DNA methyltransferase 1 (DNMT1) in LSECs elicits epigenetic reprogramming of liver ECs, resulting in a decreased number of LSECs and an increased number of macrovascular ECs. This “sinusoidal-to-macro” vascular maladaptation triggers a distinct LSEC angiocrine secretome, characterized by the secretion of insulin-like growth factor-binding protein 7 and ADAM metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1) in EVs, which promotes the recruitment of fibrogenic Th17 cells to the liver.¹⁴⁴ The above observations indicate that LSECs may regulate the immune response during MASLD by recruiting macrophages and Th17 cells.

In chronic liver diseases, a vicious cycle leads to the reciprocal exacerbation of inflammation, fibrosis, and an aberrant LSEC phenotype and angiocrine pattern. LSEC-

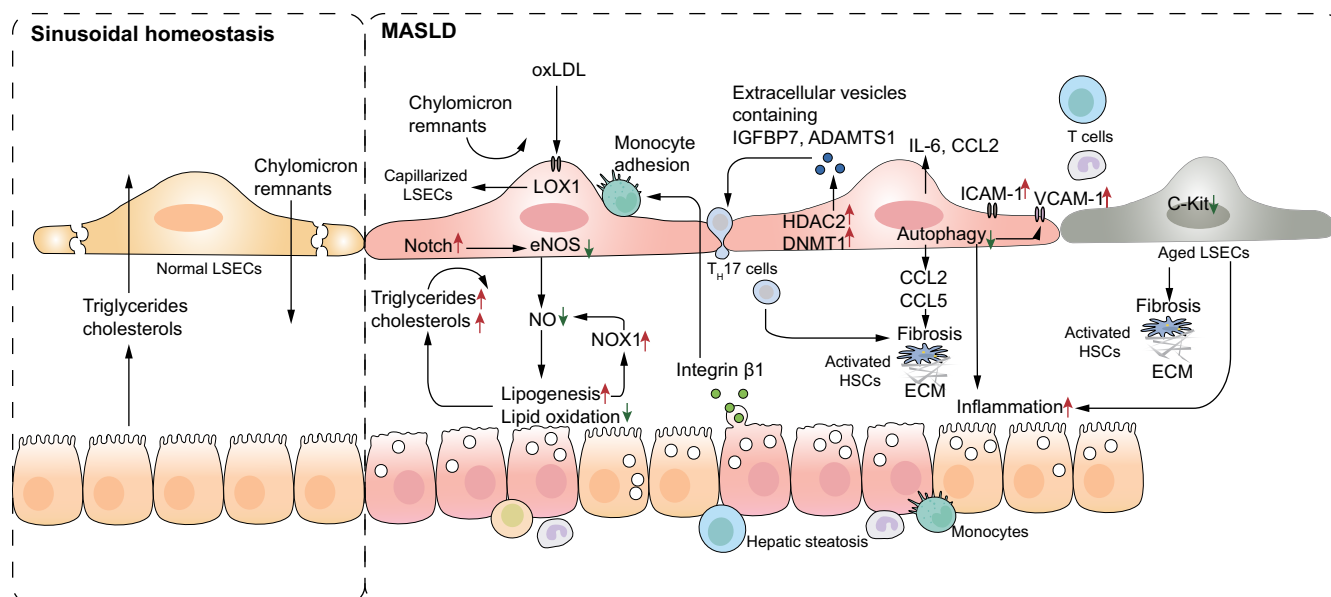


Fig. 4. The role of LSEC angiocrine signaling in MASLD. During sinusoidal homeostasis, normal LSECs contribute to the regulation of lipid synthesis and transportation. In MASLD, capillarized LSECs have decreased porosity and impeded triglyceride, cholesterol, and chylomicron remnant transportation through the sinusoid. Moreover, activation of the Notch signaling pathway in capillarized LSECs leads to decreased NO production. Reduced NO production increases lipogenesis and impairs lipid oxidation, leading to lipid accumulation in the liver. Furthermore, capillarized LSECs acquire proinflammatory phenotypes and enhance the secretion of chemokines and the expression of adhesion molecules. As a result, immune cells are recruited to the liver and activated, contributing to liver inflammation during MASLD. ECM, extracellular matrix; HSCs, hepatic stellate cells; LSECs, liver sinusoidal endothelial cells; MASLD, metabolic dysfunction-associated steatotic liver disease; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein.

specific activation of the Notch signaling pathway mediates LSEC maladaptation and inhibition of eNOS, which is associated with the aggravation of methionine- and choline-deficient diet-induced liver steatosis and inflammation in mice.¹⁴⁵ In the progression of MASLD, endothelial-derived Ang-2, a promoter of pathological angiogenesis, plays a crucial role in the inflammation-angiogenesis pathogenic amplification loop.¹⁴⁶ Nevertheless, whether LSECs or other EC compartments constitute the major source of Ang-2 in MASLD progression remains to be addressed. LSEC-mediated angiocrine signaling is also involved in the inflammation-fibrosis axis during MASLD. In patients with MASH or mice fed a high-fat diet, the autophagy of LSECs is impaired.¹⁴⁷ These defective LSECs increase CCL2, CCL5, and VCAM-1 expression and promote liver inflammation, fibrosis, and MASLD progression, probably via abnormal angiocrine signaling.¹⁴⁷ Moreover, LSEC senescence also plays a role in MASH progression. Aging-associated liver steatosis and inflammation can downregulate c-kit in LSECs, promoting LSEC senescence. Downregulated c-kit in LSECs results in dysregulated angiocrine and metabolic gene expression, which in turn exacerbates MASH-associated steatosis, fibrosis, and inflammation in mice.¹¹⁸ ScRNA-seq and cell-cell communication network analysis also revealed that with the progression of MASH, Egr1^{hi} LSECs and Ly6a^{hi} LSECs expanded and showed increased interaction with HSCs,¹⁴⁸ indicating a pro-fibrogenic role of these LSECs.

Overall, during the progression of MASLD and MASH, LSECs exhibit an abnormal phenotype and angiocrine signaling, partially contributing to lipid accumulation, immune cell recruitment and activation, and liver fibrosis. Therefore, defective LSECs are active and critical players in MASLD pathogenesis and progression. Restoring LSEC function might be a potential therapeutic target for MASLD treatment.

Autoimmune liver disease

Autoimmune liver diseases, including primary biliary cholangitis, autoimmune hepatitis, and primary sclerosing cholangitis, are chronic inflammatory diseases associated with abnormal levels of autoimmune-related serum antibodies, the exact causes of which remain unknown. It is generally accepted that adaptive immune responses to self-antigens cause autoimmune liver disease. However, the role of LSECs in autoimmune liver diseases remains largely unknown. Liver-infiltrating T cells play a pivotal role in the pathogenesis of autoimmune liver disease.¹⁴⁹ In the absence of professional antigen-presenting cells, LSECs can prime a cluster of CD4⁺ T cells that can inhibit hepatic inflammation in a chicken ovalbumin-specific autoimmune hepatitis mouse model.¹⁵⁰ Moreover, nanoparticle-mediated autoantigen peptide delivery to LSECs inhibits the activation of non-specific T cells and ameliorates CD8⁺ T cell-driven autoimmune cholangitis, indicating that drug delivery systems targeting LSECs might offer a potential therapeutic strategy for autoimmune liver disease.¹⁵¹ Given that LSECs have intimate crosstalk with immune cells infiltrating the liver and may regulate T-cell infiltration and activation in autoimmune liver disease, it is worth discovering whether dysfunctional LSECs and aberrant angiocrine signaling contribute to the loss of immune homeostasis during autoimmune liver disease.

Liver fibrosis

Liver fibrosis is characterized by excessive ECM deposition and the transformation of HSCs into myofibroblasts. When liver injury is overwhelming or persistent, the pro-regenerative function of LSECs can be significantly impaired with LSECs undergoing capillarization and acquiring a profibrotic phenotype, resulting in dysregulated sinusoidal homeostasis, maladaptive liver healing, and liver fibrosis (Fig. 5).

LSEC capillarization can occur before fibrosis develops.¹⁴ Capillarized LSECs not only lose their membrane fenestrae and ability to maintain HSC quiescence but also acquire a profibrotic phenotype. This phenotypic switch can occur in chronic liver injury and might be regulated by the FGFR1-CXCR4 signaling pathway¹¹³ or the ERK1/2-AKT signaling pathway.¹⁵² After acquiring a profibrotic phenotype, LSECs can secrete a wide spectrum of profibrotic factors. For example, in both bile duct ligation (BDL)- and CCl₄-induced liver fibrosis in mice, adipocyte fatty acid binding protein (A-FABP) potentiates LSEC capillarization via the Hedgehog signaling pathway, and capillarized LSECs in turn upregulate the secretion of A-FABP, which promotes the initiation and perpetuation of HSC activation via the JNK/cJUN-TGF- β signaling pathway.¹⁵³ VCAM-1 was previously thought to function as a leukocyte adhesion molecule. A recent study demonstrated that VCAM-1 contributed to LSEC capillarization and that VCAM-1 in LSECs is involved in the activation of HSCs and liver fibrosis through a YAP-1-dependent signaling pathway *in vitro*.¹⁵⁴ LSEC-specific *Vcam-1* depletion ameliorated CCl₄-induced liver fibrosis in mice.¹⁵⁴ Lipid-bound EVs secreted by cells promote intercellular communication by delivering specific content to target cells. Sphingosine kinase 1 (SK1) is the synthetase of sphingosine-1-phosphate (S1P), and elevated expression of both SK1 and S1P was observed in LPS-induced capillarized LSECs accompanied by liver fibrosis in a mouse model of chronic liver congestion. Inhibition of S1P receptors in these mice ameliorated liver fibrosis.¹⁵⁵ Accordingly, LSEC-derived SK1-containing EVs promote HSC activation *in vitro*.¹⁵⁶ Nevertheless, whether LSEC-derived EVs regulate liver fibrosis *in vivo* remains elusive. Other LSEC-derived profibrotic factors include endothelin-1 (ET-1), EIIIA-connected fibronectin, and regulated on activation of normal T-cell expressed and secreted (RANTES).^{152,156–159} These profibrotic factors not only induce HSC activation but also promote HSC migration and contraction. Crosstalk between hepatocytes and LSECs also contributes to fibrogenesis. Injured hepatocytes secrete leukocyte cell-derived chemotaxin 2 (LECT2), which activates Tie2-Tie1 signaling in LSECs and promotes sinusoidal capillarization and liver fibrosis in mice.¹⁶⁰ Overall, capillarized LSECs may promote liver fibrosis by acquiring a profibrotic phenotype and activating HSCs.

As healthy LSECs have antifibrotic effects, treatments to reverse LSEC capillarization are promising therapeutic strategies for liver fibrosis. In rats with thioacetamide-induced liver fibrosis, activation of soluble GC (sGC) restores LSECs to a normal phenotype.³⁷ Successively normalized LSECs restore HSC quiescence and thus block the progression and accelerate the resolution of liver fibrosis in the absence of further treatment with sGC activator.³⁷ As NO is not found to be involved in this antifibrotic process, the underlying mechanisms

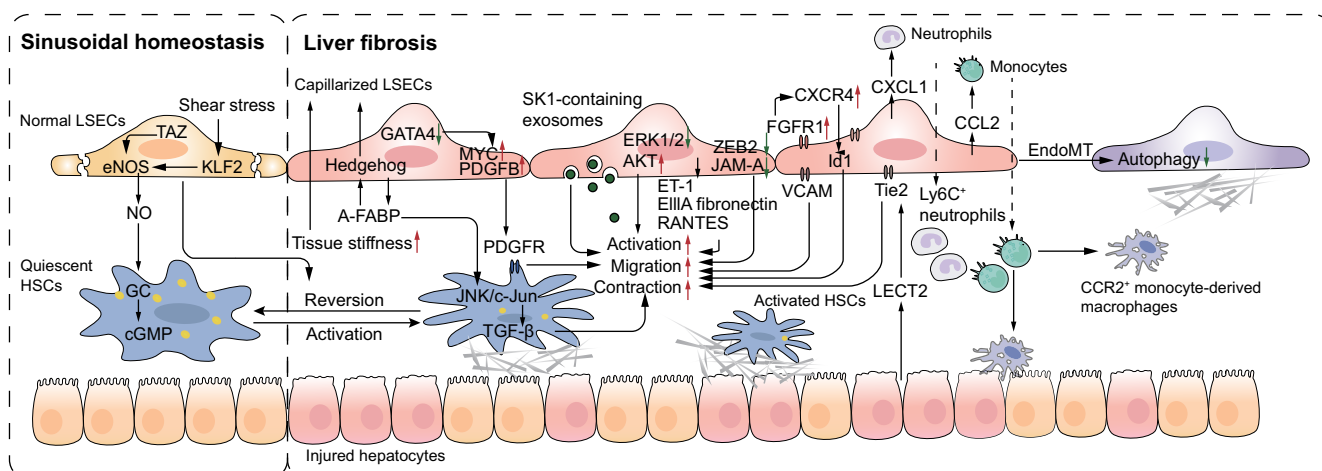


Fig. 5. The role of LSEC angiocrine signaling in liver fibrosis. During sinusoidal homeostasis, healthy LSECs sense shear stress and induce the expression of KLF2. KLF2 and TAZ can induce LSEC production of eNOS and NO. The NO-GC-cGMP axis maintains HSC quiescence. Moreover, healthy LSECs can reverse activated HSCs to a quiescent state. In contrast, during liver fibrosis, injured LSECs exhibit increased expression of CXCR4 and FGFR1 and can secrete a broad spectrum of profibrotic factors, such as A-FABP, PDGFB, SK1-containing exosomes, ET-1, and RANTES, which induce HSC activation, migration, and contraction. Injured LSECs also exhibit decreased expression of GATA4, ZEB2, ERK1/2, and JAM-A, which also facilitates liver fibrosis. Moreover, LSECs can undergo EndoMT and produce ECM, accompanied by impaired autophagy during liver fibrosis. LSECs also secrete chemokines such as CXCL1 and CCL2, which induce immune cell infiltration and lead to exaggerated liver inflammation and fibrosis. Injured hepatocytes secrete LECT2, which binds to the Tie2 receptor on LSECs and promotes HSC activation. VCAM expressed on LSECs also contributes to liver fibrosis. EndoMT, endothelial-mesenchymal transition; HSCs, hepatic stellate cells; LSECs, liver sinusoidal endothelial cells; NO, nitric oxide.

of this effect deserve further investigation. Mechanistically, shear stress-induced KLF2 is one of the most well-defined vasoprotective factors that explains the antifibrotic effect of LSECs.^{161,162} Other signaling pathways, such as GATA4 and transcriptional co-activator with PDZ-binding motif (TAZ), may also be involved. GATA4 is a transcription factor that controls LSEC specification during early liver development.¹⁶³ Endothelial GATA4 protects against perisinusoidal liver fibrosis by repressing angiocrine MYC/PDGFB/PDGFR β signaling-mediated HSC activation in mice.¹⁶⁴ LSEC-specific *Gata4* deletion results in sinusoid capillarization and profibrotic PDGFB secretion, provoking HSC activation.¹⁶⁴ TAZ, a transcription coactivator, can stimulate *Nos3* (gene for eNOS) transcription and NO production together with another vasoprotector, KLF2, thus preventing LSEC capillarization and liver fibrosis.¹⁶⁵ Presumably, treatment targeting these sinusoidal protective factors might be a potential approach for restoring the LSEC phenotype and ameliorating liver fibrosis.

One feature of liver fibrosis is an imbalance of ECM accumulation and tissue repair after liver injury. The mechanism by which LSECs selectively influence regeneration and fibrosis in the context of chronic liver diseases remains unclear. CXCL12, the ligand of both CXCR4 and CXCR7 expressed on LSECs, is upregulated during liver injury and switches between profibrotic FGFR1-CXCR4 vs. pro-regenerative CXCR7-Id1 angiocrine signaling in sinusoids.¹¹³ Endothelial-specific *Fgfr1* or *Cxcr4* depletion, as well as CXCR7 activation, facilitates pro-regenerative signaling and prevents liver fibrosis.¹¹³ This divergent angiocrine signaling pathway that promotes liver repair without causing fibrosis has potential for the clinical treatment of liver fibrosis. Similarly, other genes, such as ZEB2 and junctional adhesion molecule A (JAM-A), also play dual roles in liver fibrosis. ZEB2 is a widely expressed transcription factor and is involved in HSC activation and proliferation

in vitro.¹⁶⁶ However, LSEC-specific *Zeb2* deficiency promotes intussusceptive angiogenesis, toxin-induced LSEC capillarization, HSC activation, and liver fibrosis, indicating a context-dependent role of ZEB2 in fibrogenesis.¹⁶⁷ JAM-A is crucial for leukocyte extravasation and is a multifaceted regulator of hepatic fibrogenesis. Complete and bone marrow-specific *Jam-a* knockout promotes monocyte-derived macrophage accumulation around macrovascular cells and liver fibrosis in mice. In contrast, endothelial-specific *Jam-a* deletion induces sinusoid capillarization and exacerbates HSC activation and liver fibrosis, while leukocyte infiltration and adhesion remain unchanged.¹⁶⁸ The versatile roles of certain genes in LSEC-associated liver fibrogenesis might depend on the sub-clusters and zonation of LSECs. Thus, further investigations, including scRNA-seq and spatial omics studies, are imperative for understanding sub-clusters and zonation of LSECs. Such insights are also crucial for discovering effective therapeutic targets for liver fibrosis.

ECM and tissue stiffness influence the LSEC phenotype.¹² This may be explained by the mechanosensing function of LSECs, as well as the manifold soluble mediators that are bound and retained by ECM fibrils, the so-called liver matrix-some.¹⁶⁹ *In vitro* studies revealed that LSECs cultured on an interstitial collagen matrix present decreased porosity.¹⁷⁰ Furthermore, along with massive ECM accumulation, the stiffness of the fibrotic liver is increased.^{42,171} LSECs are sensitive to mechanical force, and when subjected to increased tissue rigidity *in vitro*, they become defenestrated within 24 h of being seeded.¹⁷² More importantly, elevated stiffness triggers an altered angiocrine gene expression profile in LSECs, including the expression of profibrotic factors,⁸¹ indicating that LSECs might acquire a profibrotic phenotype through mechanosensing. In addition, LSECs can also produce and deposit ECM components by undergoing endothelial-mesenchymal

transition (EndoMT) in liver fibrosis.^{147,173–175} EndoMT allows LSECs to acquire a phenotype similar to that of myofibroblasts.^{173,174} It was suggested that EndoMT might be triggered by impaired autophagy in LSECs,¹⁴⁷ and EndoMT can be partially prevented by BMP-7.¹⁷⁵ Moreover, pseudotime analysis of the scRNA-seq data revealed that EndoMT might occur after LSEC capillarization.¹⁷³ The eNOS-sGC activator YC-1, which can abolish capillarization, could also block EndoMT in a CCl₄-induced murine liver fibrosis model.¹⁷³ However, the percentage of LSECs that undergo EndoMT and the extent to which they contribute to liver fibrosis remain unclear and need to be precisely determined in the future.

LSECs are also the gatekeepers of hepatic immunity. LSECs exhibit increased immunogenicity in the fibrotic liver and trigger the recruitment and activation of immune cells.¹⁷⁶ Mass cytometry by time-of-flight was used to determine the accumulation of CCR2⁺ monocyte-derived macrophages and Ly6C⁺ neutrophils in fibrotic murine livers.⁵⁸ CCL2 is a ligand for CCR2 and a potent monocyte chemokine. CCL2 is predominantly produced by LSECs in the context of liver fibrosis.⁵⁸ LSEC-specific *Ccl2* deficiency attenuates hepatic macrophage accumulation.⁵⁸ Neutrophil infiltration during liver fibrosis is predominantly induced by LSEC-secreted CXCL1.^{80,81}

Collectively, dedifferentiated LSECs and loss of sinusoidal homeostasis exaggerate liver fibrosis, and in turn, liver fibrosis maintains the profibrotic phenotype of LSECs. Notably, in a mouse model of CCl₄-induced liver fibrosis, LSECs in zone 3 exhibited the most significantly altered gene expression profile.^{3,177} Distinctly, in a mouse model of Amylin diet-induced MASH, LSECs in all zones present similar gene expression

changes,¹³⁴ indicating etiology-specific pathogenic alterations of LSECs in toxic vs. metabolic injury. Thus, identifying molecular and functional heterogeneity is important for elucidating the specific role of LSECs in liver fibrosis induced by different pathogenic stimuli.

Portal hypertension

One of the major functions of LSECs is regulating the vascular tone of the hepatic microcirculatory system. Increased sinusoidal pressure might dampen the mechanosensing function of LSECs.¹⁷⁸ The mechanical force sensor on LSECs can detect the shear stress of the blood flow and balance the vascular tone accordingly by secreting vasodilators or vasoconstrictors, such as NO, eicosanoids, ET-1, and carbon monoxide.^{179–181} Disorders of LSEC function and LSEC capillarization cause a significant increase in intrahepatic vascular resistance and contribute to portal hypertension (Fig. 6). Portal hypertension is one of the most life-threatening complications of cirrhosis. Accumulating evidence has revealed the contribution of LSECs to the progression of portal hypertension.^{15,182,183} The transcription factor GATA4 controls LSEC specification during early liver development, and GIMAP5 is upstream of GATA4. ScRNA-seq analysis of an LSEC-specific *Gimap5*-deficient mouse model revealed increased LSEC capillarization and portal hypertension.¹⁸⁴

In the case of liver injury, LSECs regulate microvascular blood flow by changing the expression of vasoconstrictors and vasodilators. ET-1 is a vasoconstrictor that may increase portal pressure during pathological conditions. Overexpression of ET-1 and cysteinyl-leukotrienes in LSECs from the cirrhotic liver

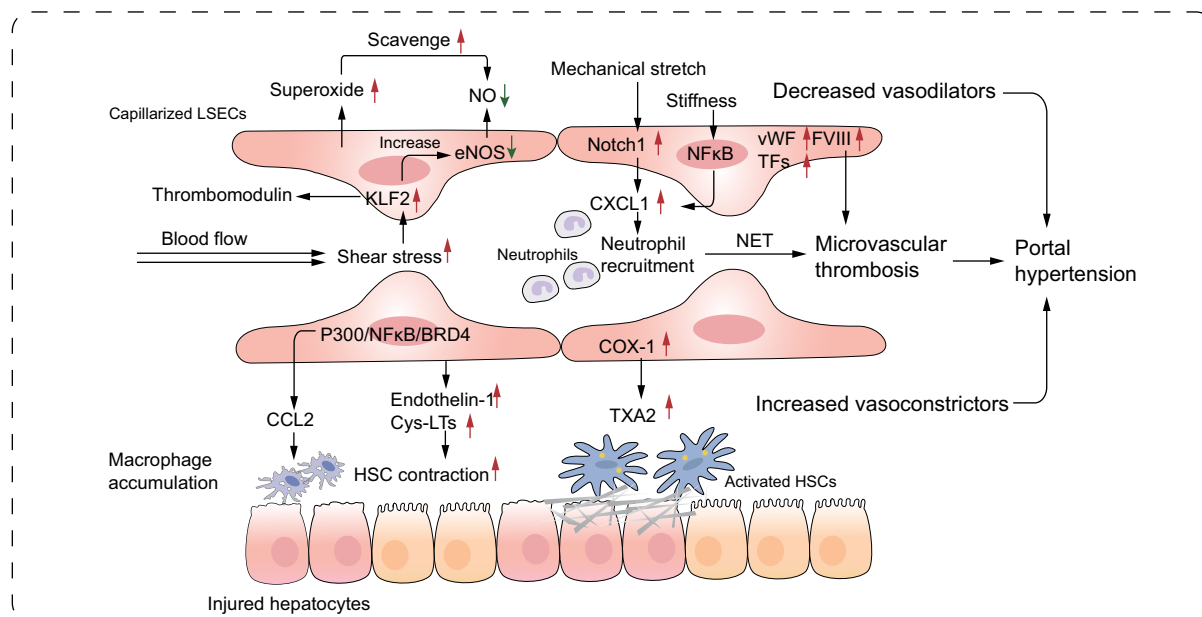


Fig. 6. The role of LSEC angiocrine signaling in portal hypertension. LSECs are involved in regulating the vascular tone of the hepatic microcirculatory system. On the one hand, injured LSECs overexpress vasoconstrictors, which induce HSC contraction. On the other hand, the release of the vasodilator NO was decreased in injured LSECs due to reduced production and increased scavenging. Consequently, intrahepatic vascular resistance and shear stress increase, leading to the upregulation of the vasoprotective factor KLF2. Increased tissue stiffness upregulates CXCL1 secretion in LSECs. CXCL1 contributes to neutrophil recruitment, which accelerates microvascular thrombosis together with LSEC overexpression of prothrombotic factors. Elevated shear stress also leads to CCL2 secretion and macrophage accumulation through the interaction of endothelial p300 with NFκB and BRD4. Decreased vasodilators, increased vasoconstrictors, and microvascular thrombosis together contribute to portal hypertension. HSCs, hepatic stellate cells; LSECs, liver sinusoidal endothelial cells; MASLD, metabolic dysfunction-associated steatotic liver disease; NET, neutrophil extracellular trap; NO, nitric oxide.

has also been observed and might be associated with enhanced HSC contraction and microvascular resistance.^{185,186} On the other hand, the release of the vasodilator NO is significantly decreased in LSECs from cirrhotic rat liver, partly due to impaired eNOS function.^{187,188} In addition to downregulated NO synthesis, increased NO scavenging by superoxide from the sinusoidal niche is also responsible for the disordered vascular tone.¹⁸⁹ Additionally, there is also crosstalk between NO and cyclooxygenases (COXs). Inhibition of COX-2 and administration of the antioxidant tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) reduces hepatic superoxide levels, thus restoring NO bioavailability in LSECs and reducing portal pressure in cirrhotic rats.^{189,190} For COX-1, primary LSECs isolated from CCl₄-induced cirrhotic rats show increased expression of COX-1 and its downstream vasoconstrictor prostanoid thromboxane A₂. Moreover, the inhibition of COX-1 and thromboxane A₂ receptors significantly reduces portal pressure in cirrhotic rats.¹⁹¹ These studies show that LSECs enhance portal hypertension by increasing vasoconstrictor and reducing vasodilator expression.

It seems that the NO synthesis signaling pathway might serve as a promising target for the clinical treatment of portal hypertension. Indeed, statins, classic cholesterol-lowering drugs, have been repurposed for the treatment of portal hypertension. The transcription factor KLF2 transcriptionally regulates various endothelial vasoprotective genes. KLF2 upregulation is induced by elevated shear stress during portal hypertension, and further activation of KLF2 by simvastatin is accompanied by an increase in vasoprotective factors, such as eNOS and thrombomodulin.^{39,161} Another drug repurposed for portal hypertension is the anticoagulant rivaroxaban. Oral rivaroxaban treatment ameliorates endothelial dysfunction and inhibits HSC activation in liver sinusoids in CCl₄- and thioacetamide-induced cirrhotic rat models.¹⁹² Consistent with these findings, Hernández-Gea *et al.* suggested that LSEC-associated endothelial dysfunction characterized by a unique genetic profile enriched in coagulation and lipid metabolism might drive porto-sinusoidal vascular disease, a rare cause of portal hypertension.¹⁹³ Moreover, an agonist of the bile acid receptor FXR (farnesoid X receptor), which has been tested as a potential pharmacological approach for treating different liver diseases,¹⁹⁴ has also shown promising effects on portal hypertension. The non-steroidal FXR agonist PX20606 upregulates eNOS and dimethylarginine dimethylaminohydrolase 1 (an enzyme that promotes NO production) in human LSECs *in vitro*.¹⁹⁵ Furthermore, *in vivo* application of PX20606 ameliorates both cirrhotic and non-cirrhotic portal hypertension in rat models. This effect might be partly attributed to decreased intrahepatic vascular resistance and restored sinusoidal function.¹⁹⁵ It seems that drugs that can regulate vasoconstrictors or vasodilators can be repurposed for the treatment of portal hypertension.

Additionally, the crosstalk between LSECs and immune cells in the sinusoid also contributes to the pathogenesis of portal hypertension. Partial ligation of the inferior vena cava (pIVCL) model mimics Budd-Chiari syndrome and induces liver venous congestion. Evident sinusoidal thrombosis, neutrophil recruitment, and portal hypertension are observed in pIVCL mouse livers.⁸⁰ Neutrophil recruitment is predominantly induced by LSEC-secreted CXCL1. Then, the recruited neutrophils can interact with platelets and promote microvascular thrombosis

via the formation of neutrophil extracellular traps, leading to increased microvascular resistance and portal pressure.⁸⁰ This angiocrine signaling by LSECs might be regulated by mechanical stretch-induced Notch1 and stiffness-induced NF- κ B translocation into the nucleus.^{80,81} Consistently, LSEC-specific *Hk2* knockout mice, a model with inhibited LSEC glycolysis, exhibit decreased CXCL1 secretion and subsequent neutrophil recruitment and amelioration of portal hypertension after pIVCL.⁸¹ Similarly, LSEC-specific *p300* deletion attenuates portal hypertension in pIVCL and CCl₄ mouse models, which is related to reduced monocyte/macrophage infiltration and HSC activation.⁵⁸ Indeed, endothelial *p300* interacts with NF- κ B and BRD4 in the *CCL2* promoter and enhancer to increase *CCL2* expression, leading to macrophage accumulation.⁵⁸

In summary, sinusoidal homeostasis is altered during portal hypertension due to dysregulated levels of vasoconstrictors, vasodilators, procoagulants, and chemokines. Thus, a drug delivery system targeting LSECs and LSEC-mediated angiocrine signaling might be a potential therapeutic strategy for the clinical treatment of portal hypertension.

Portal vein thrombosis

LSECs represent the primary contact with oxygen and nutrient-rich blood from the hepatic artery and portal vein in the liver.¹⁹⁶ Healthy LSECs inhibit thrombosis by generating eNOS-derived NO. Consistently, tetrahydrobiopterin (BH₄) partly restores intrahepatic endothelial function by activating the eNOS-NO signaling pathway.¹⁹⁷ The incidence of portal vein thrombosis (PVT) has been demonstrated to be significantly associated with cirrhosis and some liver diseases. According to a meta-analysis of prospective and retrospective cohort studies, the risk of PVT is associated with reduced portal venous blood flow.¹⁹⁸ As LSEC-mediated angiocrine signaling can regulate portal venous blood flow, it is presumed that LSEC-mediated angiocrine signaling might also be involved in the development of PVT.¹⁹⁹

Chronic liver inflammation or injury is also a pro-thrombotic condition, because disease-related stimuli may lead to dysfunction and capillarization of LSECs, which upregulate pro-thrombotic von Willebrand factor, factor VIII, tissue factors, and fibrinogen-like protein 2, among others.^{200–203} The vasoprotective transcription factor KLF2 in LSECs sustains sinusoidal homeostasis, which is a crucial process for preventing thrombi.³⁹ Capillarized LSECs lose their antithrombotic function due to decreased expression of KLF2, which further promotes the recruitment and activation of platelets and results in the generation of microthrombi and fibrins.⁹ Moreover, disturbed sinusoidal homeostasis also accelerates thrombosis. The interaction between LSEC-recruited neutrophils and platelets plays a pivotal role in the formation of neutrophil extracellular traps, which induce microvascular thrombosis.⁸⁰ Therefore, LSECs play an important role in thrombosis during liver injury, and restoring LSEC function and sinusoidal homeostasis might serve as a potential anticoagulant therapeutic target.

Therapeutic targets

LSECs are the first cells in contact with injury factors from the portal vein, and LSEC-mediated angiocrine signaling is the critical process for maintaining sinusoidal homeostasis. Thus,

therapies targeting dysfunctional LSECs and angiocrine signaling may be able to modulate this process and interrupt liver disease progression. The emerging therapeutic targets of LSECs for liver diseases, including VEGF/VEGFR2, Notch-eNOS/sGC, ERK/MMPs, and COXs, have been described in the previous sections and are summarized in Table 1.

The development of pharmacological therapies that target LSECs are still in the preclinical phase. As such, there are no clinical trials involving LSECs as therapeutic targets. However, repurposing existing drugs related to LSEC-mediated angiocrine signaling, such as sorafenib, anlotinib, and celecoxib, might be a highly effective approach for the clinical treatment of liver diseases.

Conclusions and prospects

LSECs display a highly specialized morphology and exclusive location within the liver, which renders them the central cells of sinusoidal homeostasis and the hub of angiocrine signaling. The intimate crosstalk within the sinusoidal niche participates in hepatocyte replication and metabolism, HSC activation and deactivation, immune cell recruitment and transmigration, etc.

Consequently, these cellular interactions promote or hamper liver regeneration and the pathogenesis of a broad spectrum of liver diseases. Although our knowledge of LSECs has expanded extensively, much effort still needs to be made to expand our understanding of LSECs. First, the injury, renewal, senescence, and death of LSECs contribute substantially to the pathogenesis of liver diseases, mainly through the altered secretion of angiocrine factors. However, the mechanisms and key regulators underlying this phenotypic switch are not fully understood and warrant further exploration. Second, dysregulated hepatic EC-mediated angiocrine signaling leads to an imbalance of regeneration, inflammation, and fibrosis. Hepatic ECs are heterogeneous cell clusters comprising arteries, veins, and capillaries. Even within the LSEC subgroup, there is considerable heterogeneity of angiocrine factors and functions in different zones. Clarifying the heterogeneity of hepatic ECs in regeneration, inflammation, and fibrosis may help in understanding the pathogenesis of most chronic liver diseases. Additionally, standardized isolation methods and *in vitro* culture protocols for sinusoidal cells are warranted to avoid discrepancies among different groups. Third, hemodynamic

Table 1. Therapies targeting LSECs for liver diseases.

Therapeutic target	Treatment	Result	Animal model	Ref
VEGF/VEGFR pathway				
VEGFR2	Sorafenib	Decreased neovascularization, attenuated hypertension, improved liver damage and fibrosis.	Portal hypertensive and cirrhotic rats	204
VEGFR2	CXCL9	Lower microvascular perfusion, reduced HSC activation, attenuated liver fibrosis.	CCl ₄ cirrhotic mice	205
VEGFR2	Anlotinib	Inhibited tumor cell migration and invasion, effective antitumor effect and low drug toxicity.	intrahepatic cholangiocarcinoma patient-derived tumor xenograft mice model	206
VEGF	Adenoviral overexpression	Increased tissue repair and fibrosis resolution. Increased CXCL9 and MMP13.	CCl ₄ withdrawal model mice	207
VEGF	Bevacizumab	Decreased liver fibrosis, sinusoidal capillarization and the number of central vessels	CCl ₄ cirrhotic mice	208
Notch-eNOS/sGC pathway				
sGC	YC-1	Reverted LSEC dedifferentiation, improved hepatocyte proliferation	CDH5-CreERT-NIC mice, CCl ₄ induced liver fibrosis	119
eNOS	AVE 9488	Lowered Portal pressure, hepatic portal-vascular resistance and perfusion pressure.	BDL rats	209
ERK/MMP pathway				
ERK1/2	Honokiol loaded nanoparticles	Maintained LSEC differentiation, improved pro-regenerative response and alleviated fibrosis.	CCl ₄ cirrhotic mice	152
MMPs	2-[(4-biphenylsulfonyl) amino]-3-phenyl-propionic acid	Attenuated ALT levels and liver micro-circulatory dysfunction.	Acetaminophen-induced mice liver injury model	210
MMP-9	MMP-9 antisense oligonucleotides	Accelerated liver regeneration, prevented IRI, reduced necrosis.	Ischemia-reperfusion rat model	211,212
COX pathway				
COX2	Celecoxib and octreotide	Attenuated liver fibrosis, reduced portal hypertension, inhibited angiogenesis.	TAA cirrhotic rats	213
COX2	Celecoxib	Relieved liver fibrosis, relieved vascular resistance, reduced hepatic oxidative stress	TAA cirrhotic rats	190
COX1	COX1 siRNA delivery to liver sinusoidal endothelium	Downregulation of TXA ₂ , attenuated portal hypertension, lowered spleen size.	CCl ₄ cirrhotic mice	214

LSECs, liver sinusoidal endothelial cells; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; HSC, hepatic stellate cell; CXCL9, C-X-C motif chemokine ligand 9; CCl₄, carbon tetrachloride; eNOS, endothelial nitric oxide synthase; sGC, soluble guanylate cyclase; BDL, bile duct ligation; ERK, extracellular signal-regulated kinase; MMP, matrix metalloproteinase; ALT, alanine aminotransferase; COX, cyclooxygenase; IRI, ischemia-reperfusion injury; TAA, thioacetamide; TXA₂, thromboxane.

shear stress and mechanical forces promote LSEC-mediated angiocrine signaling. *In vivo* studies and preclinical models are encouraged to better consider the intrahepatic milieu, hemodynamic shear stress, and matrix-derived mechanical forces on LSECs. As mechanical forces and increased stiffness epigenetically regulate LSEC-mediated angiocrine signaling, studies on histone modifications may help to explore the mechanism of this critical process. Finally, the liver receives blood from the gastrointestinal tract and is a hub that connects to various tissues. The role and mechanism of LSEC-mediated angiocrine signaling in the crosstalk between the liver and other organs should also be identified. As the liver governs the metabolism of glucose, amino acids, fatty acids, drugs, and hormones, LSEC-mediated angiocrine signaling may also

connect to various tissues and be metabolically involved in the pathogenesis of liver diseases. Although many challenges exist, the future of LSEC research seems promising with the support of a growing number of devoted teams and quickly evolving technologies, including single-cell technologies, spatial multi-omics, lineage tracing, epigenetic modification-related techniques, liver-on-chip models, and liver organoids.

In conclusion, LSEC-mediated angiocrine signaling in normal livers maintains sinusoidal homeostasis by leading to crosstalk among LSECs and other liver cells, such as hepatocytes, HSCs, and immune cells. Aberrant angiocrine signaling is involved in liver regeneration and the pathogenesis of several liver diseases. Therapeutic agents targeting LSECs and angiocrine signaling may shed light on the treatment of liver diseases.

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Abbreviations

AH, alcohol-associated hepatitis; ALD, alcohol-related liver disease; Ang-2, angiopoietin-2; BDL, bile duct ligation; BMP, bone morphogenetic protein; BRD4, bromodomain containing 4; CCL, C-C motif chemokine ligand; CC14, carbon tetrachloride; CCR2, C-C motif chemokine receptor 2; COXs, cyclooxygenases; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; EC, endothelial cell; ECM, extracellular matrix; EndoMT, endothelial-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; EVs, extracellular vesicles; FGFR1, fibroblast growth factor receptor 1; (s)GC, (soluble) guanylate cyclase; HGFs, hepatocyte growth factors; HSCs, hepatic stellate cells; ICAM-1, intercellular cell adhesion molecule-1; IL-, interleukin-; KC, Kupffer cells; KLF2, Kruppel-like factor 2; LPS, lipopolysaccharide; LSECs, liver sinusoidal endothelial cells; MASLD, metabolic dysfunction-associated steatotic liver disease; NO, nitric oxide; PDGF(R)B, platelet-derived growth factor subunit B (receptor); pIVCL, partial ligation of the inferior vena cava; PVT, portal vein thrombosis; scRNA-seq, single-cell RNA sequencing; STAT3, signal transducer and activator of transcription 3; TGF- β , transforming growth factor- β ; TNF α , tumor necrosis factor- α ; VAP1, vascular adhesion protein 1; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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Conflict of interest

FT: grants or contracts from any entity-AstraZeneca, MSD, Gilead; consulting fees-Allergan, AstraZeneca, Gilead, GSK, AbbVie, Alnylam, BMS, Intercept, Inventiva, Pfizer, Novartis, Novo Nordisk, MSD, Sanofi; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events-Gilead, AbbVie, Falk, Merz, Intercept, Sanofi, Astra Zeneca; support for attending meetings and/or travel-Gilead; participation on a Data Safety Monitoring Board or Advisory Board-Sanofi, Pfizer. EK: grants or contracts from any entity-National Institute of Health, Gilead; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events-Honoraria for a lecture at Pittsburgh Liver Research Center; patents planned, issued or pending-Patent application number: 63/185,895; leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid-AASLD committee member, NIH study section. The other authors have no conflicts of interest in relation to this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

JG, VS, FT, and CT designed and coordinated the study; JG, TL, EK, EL, and YG wrote the manuscript; JG, TL, EK, and YG finalized the figures; JG, TL, EK, AG, BD, FT, CT, and VS revised and finalized the manuscript.

Supplementary data

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